For. Path. 31 (2001) 53–63

ISSN 1437–4781

Mycosphaerella species occurring on *Eucalyptus globulus* and *Eucalyptus nitens* plantations of Tasmania, Australia

By A. W. Milgate[1,3], Z. Q. Yuan[3], R. E. Vaillancourt[1,3] and C. Mohammed[2,3,4]

[1]School of Plant Science, University of Tasmania, GPO Box 252–55, Hobart 7001, Tasmania; E-mail: amilgate@utas.edu.au; [2]School of Agricultural Science, University of Tasmania, GPO Box 252–54, Hobart 7001, Tasmania; [3]CRC for Sustainable Production Forestry, GPO Box 252–12, Hobart 7001, Tasmania; [4]CSIRO Forestry and Forest Products, GPO Box 252–12, Hobart 7001, Tasmania

Summary

The genus *Mycosphaerella* Johanson contains many pathogens capable of causing a severe impact on the growth of susceptible eucalypt species. The lack of knowledge about which species are present in Tasmania and their potential risk to the plantation industry prompted this study into the *Mycosphaerella* species occurring on *Eucalyptus globulus* and *Eucalyptus nitens* plantations in Tasmania. A total of 36 plantation and five road verge sites of *E. globulus* and *E. nitens* were sampled. Five *Mycosphaerella* species and three species from associated anamorph genera were isolated and identified in Tasmania; *Mycosphaerella nubilosa*, *Mycosphaerella cryptica*, *Mycosphaerella tasmaniensis*, *Mycosphaerella grandis*, *Mycosphaerella vespa*, *Coniothyrium ovatum*, *Sonderhenia eucalypticola* and *Sonderhenia eucalyptorum*. The most frequently isolated species with the highest incidence and severity of infection were *M. cryptica* and *M. nubilosa*. These two species appear to have the greatest potential to damage juvenile eucalypt plantations in Tasmania. A link between *Mycosphaerella vespa* and *Coniothyrium ovatum* is described for the first time.

1 Introduction

Australia has a strong commitment at both an international and domestic level to the role of forestry in sustainable development. The forest industry in Australia is therefore increasingly applying intensive silvicultural regimes to timber production, involving thinning in native forests and establishment of plantations. Of the approximately 200 000 ha of eucalypt plantations established in Australia to date, most are intended for production of pulpwood on short rotations of 15–20 years.

Between 1991, and, 1996 in Tasmania more than 7000 hectares of *Eucalyptus nitens* (Deane and Maid.) Maid. plantations were established for saw-log or veneer on rotations of 20-plus years. The Tasmanian Regional Forest Agreement signed in November 1997 by the Federal and State Governments has provided further funding for solid wood plantation development to compensate for losses of production forest to reserves. Under it, another 20 000 hectares will be planted with *Eucalyptus globulus* Labill. as well as *E. nitens* over the next 5 years.

Research in Tasmania focuses on developing management strategies to avoid or reduce the impact of fungal pathogens in these high cost input plantation systems. Serious *Mycosphaerella* defoliation events in native and regrowth eucalypts have been associated with wet and humid conditions frequently prevalent in Tasmania, especially the north-western region, a centre for the expanding plantation industry in Tasmania. The recent

Received: 11.5.2000; accepted: 3.8.2000; editor: P. Raddi

identification of a new species, *Mycosphaerella tasmaniensis* Crous and M. J. Wingf. (CROUS et al. 1998) pathogenic on *E. nitens* provided further impetus to investigate *Mycosphaerella* species in Tasmania.

Many *Mycosphaerella* species are well documented pathogens of *Eucalyptus* foliage especially in countries where eucalypts are propagated for commercial wood production (GANAPATHI and CORBIN 1979; PARK and KEANE 1984; CARNEGIE and KEANE 1994; CROUS and WINGFIELD 1996; CROUS and WINGFIELD 1997; CROUS 1998). Currently 30 species of *Mycosphaerella* have been described from around the world on *Eucalyptus* foliage and 13 of these species are found in Australia. Seven species have been observed in Tasmania on a range of commercial and noncommercial eucalypt species (PARK and KEANE 1984; CROUS et al. 1998, CARNEGIE, personal comm. 1998). This study is however, the first systematic attempt to identify the *Mycosphaerella* species infecting young eucalypt plantations in Tasmania.

The taxonomy of *Mycosphaerella* species is complex and requires a high level of expertise to differentiate between them. This has created a situation where the re-examination of leaf blotch symptoms previously attributed to a single *Mycosphaerella* species has revealed the involvement of two or more species (CROUS and WINGFIELD 1996). Differences between species are often slight and not discernible at a macro level, such as the lesion, and a detailed examination of microscopic morphology is required. Thus it is important to re-visit older records of *Mycosphaerella* species in Australia, given the large number of newly described species on eucalypts from overseas. This article presents the results of a survey of *Mycosphaerella* species mainly on the juvenile foliage of plantation *E. globulus* and *E. nitens* in Tasmania, Australia.

2 Materials and methods

Eucalyptus globulus and *E. nitens*, the two main plantation species in Tasmania were selected as the focus for the survey. Plantations up to 4 years old were chosen in sites that were distributed across the major growing areas within Tasmania (Fig. 1). Collections were made at 36 plantations (27 in northern Tasmania and nine in south-eastern Tasmania). Young *E. globulus* wildlings were sampled at five roadside sites in eastern and southern Tasmania. Sampling was carried out within a plantation site by collecting necrotic juvenile and intermediate leaves displaying symptoms consistent with *Mycosphaerella* leaf blotch (MLB) approximately every 20 m along an 80 m transect. Additionally a brief survey was conducted of trees in proximity to the transect that displayed any MLB symptoms other than those observed at the transect sampling points. The MLB severity (percentage of a trees leaf surface area that is necrotic) was estimated for each species and for each tree sampled. An average MLB severity was calculated for each site sampled.

The lesions on the leaves from each sample were examined and measured. Specimens of fungal fruiting bodies were prepared for microscopic examination as squash mounts and median thin sections, stained with Lactophenol Cotton Blue, Ammoniacal Congo Red or mounted in distilled water or glycerol. Wherever possible 30 measurements were made of morphological structures and their extremes given in parentheses. Specimens were lodged at the Herbarium of the Institute for Horticultural Development in Melbourne, Australia (VPRI) (Table 1).

Single ascospore cultures were established on 2% malt extract agar (MEA) using the technique described by CROUS, WINGFIELD and PARK (1991). After 24 h the germinating spores were transferred onto fresh plates of 2% MEA and incubated in the dark at 20°C. The pattern of spore germination was also recorded and photographed at 24 h. For any one site isolations were attempted from up to five lesions of similar morphology. If more than one lesion type was identified further isolations were carried out for up to five

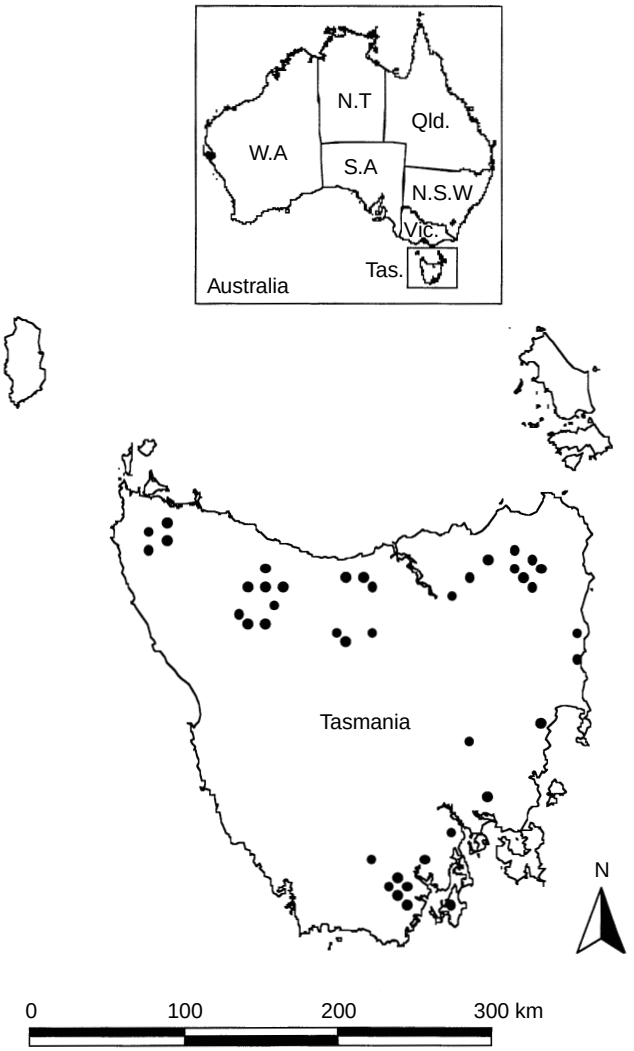


Fig. 1. Location of sites sampled around Tasmania during a survey of *E. globulus* and *E. nitens* for *Mycosphaerella* species

lesions of each additional lesion type. Using this method it was possible to determine the *Mycosphaerella* species present at each site and calculate the percentage of sites at which a particular *Mycosphaerella* species was found.

Connections of teleomorph and anamorph were carried out by single ascospore culture method.

Table 1. **Samples deposited in the Herbarium of the Institute for Horticultural Development in Melbourne, Australia (VPRI)**

Species	Host species	Location[1]	Date	Collector	VPRI
M. cryptica	*E. nitens*	Woolnorth	2 Jun. 1998	A. W. Milgate	22383
M. grandis	*E. nitens*	West Ridgely	17 Dec. 1997	A. W. Milgate	22380b
M. nubilosa	*E. globulus*	Levondale	16 Apr. 1997	A. W. Milgate	22384a
M. tasmaniensis	*E. globulus*	Franklin Rivulet	20 Jun. 1997	A. W. Milgate	22382
M. tasmaniensis	*E. nitens*	West Ridgely	17 Dec. 1997	A. W. Milgate	22380a
M. tasmaniensis	*E. nitens*	West Ridgely	23 Jan. 1998	A. W. Milgate	22381
M. vespa	*E. globulus*	Scamander	16 Sep. 1998	A. W. Milgate	22379a
M. vespa	*E. globulus*	Bruny Island	20 Jan. 1998	C. Mohammed	22378a
M. vespa	*E. globulus*	Swansea	27 May 1998	A. W. Milgate	22377
M. vespa	*E. globulus*	Hobart	9 Apr. 1998	A. W. Milgate	22388
C. ovatum	*E. globulus*	Scamander	16 Sep. 1998	A. W. Milgate	22379b
C. ovatum	*E. globulus*	Bruny Island	20 Jan. 1998	C. Mohammed	22378b
S. eucalypticola	*E. globulus*	West Ridgely	17 Dec. 1997	A. W. Milgate	22385
S. eucalypticola	*E. globulus*	Levondale	16 Apr. 1997	A. W. Milgate	22384b

[1]All locations within Tasmania, Australia.

3 Results

3.1 Survey

Mycosphaerella cryptica (Cke.) Hansf. was the most commonly isolated *Mycosphaerella* species in Tasmanian eucalypt plantations (Table 2). It was isolated from 42% of *E. globulus* sites sampled for the survey and from 35% of *E. nitens* sites. The severity of this pathogen at the sites was variable ranging from less than 3% to 20–30% of leaf surface area necrotic.

Mycosphaerella nubilosa (Cke.) Hansf. was isolated from 42% of *E. globulus* sites surveyed. The severity of this pathogen on *E. globulus* was highly variable ranging from less than 3% to greater than 75% of leaf surface area necrotic. *Mycosphaerella nubilosa* was not isolated from *E. nitens* during this survey.

Table 2. **Percentage of sites where *Mycosphaerella* species and their anamorphs were found on *E. globulus* (24 sites) and *E. nitens* (17 sites) in Tasmania**

Mycosphaerella species	*E. globulus* (%)	*E. nitens* (%)
M. cryptica	42	35
M. nubilosa	42	0
M. tasmaniensis	4	12
M. grandis	8	35
[1]*M. vespa*	21	0
[1]*Coniothyrium ovatum*	21	0
Sonderhenia eucalypticola	21	0
Sonderhenia eucalyptorum	4	0
No *Mycosphaerella* sp. present	8	35

[1]*Mycosphaerella vespa* was identified at one plantation site and four roadside sites. *Coniothyrium ovatum* was identified at five roadside sites.

Mycosphaerella tasmaniensis was isolated from three plantation sites with high levels of infection, severity ranged from 30% to greater than 70% of leaf surface area necrotic. The anamorph of *M. tasmaniensis*, *Mycovellosiella tasmaniensis* Crous and M. J. Wingf. was not observed from the field collections. However it was produced in culture from single ascospore isolates, although not all isolates produced conidia and often those that did produce conidia did not re-produce conidia when subcultured.

Mycosphaerella grandis Carnegie and Keane was frequently found associated with older lesions of *M. tasmaniensis*, *M. nubilosa* and *M. cryptica*. *Mycosphaerella grandis* was isolated from 20% of all sites sampled. It was found with greater frequency at *E. nitens* sites than *E. globulus* sites (35 to 8%, respectively).

Mycosphaerella vespa Carnegie and Keane was present at five *E. globulus* sites in eastern and southern Tasmania. Only one of these locations was from a plantation site; the other four collection sites being road verges. It was present on both adult and juvenile foliage. *Coniothyrium ovatum* Swart was not found at any plantation sites surveyed but was present at five roadside *E. globulus* sites.

Sonderhenia eucalypticola Swart and Walker and *Sonderhenia eucalyptorum* (Hansf.) Swart and Walker were isolated only from *E. globulus* plantations. *Sonderhenia eucalypticola* was identified at 21% of *E. globulus* sites, however, the severity was low, with a maximum of 3–5% of leaf surface area being necrotic. *Mycosphaerella walkeri* Park and Keane the teleomorph of *S. eucalypticola* was not isolated. *Sonderhenia eucalyptorum* was found at only one site and with very low severity.

3.2 Taxonomy

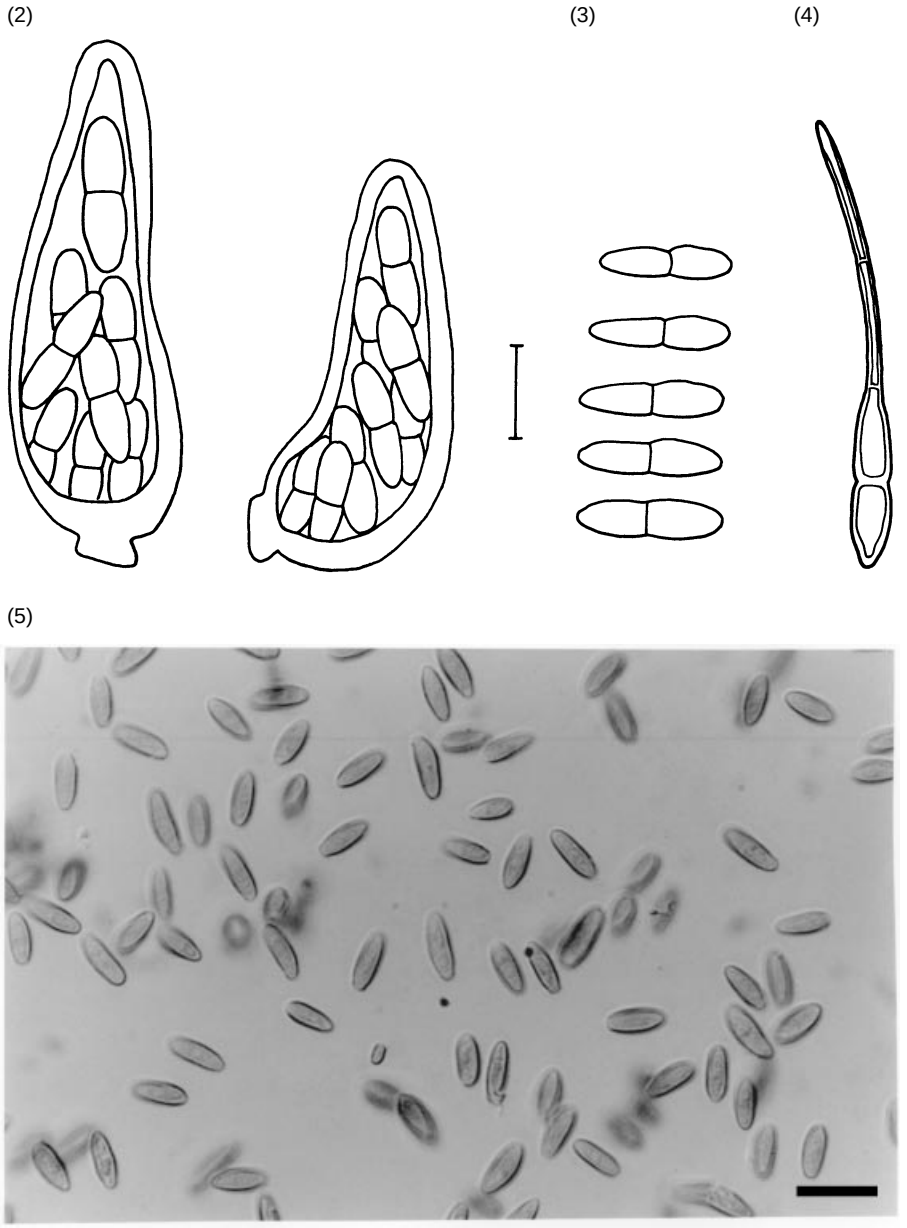
The Tasmanian fungal collections showed little variation in symptoms and morphology from other published descriptions. Table 3 compares measurements from this study with those from relevant literature.

The morphology of *M. vespa* and *C. ovatum* (Figs 1–5), species linked by this study, are described in detail; *Mycosphaerella vespa* Carnegie and Keane, Mycological Research 102: 1274–1276 (1998); Anamorph: *Coniothyrium ovatum* H. J. Swart, Transactions of the British Mycological Society 86: 495–496 (1986).

Lesions circular to irregular, amphigenous, up to 7 mm diameter, rarely coalescing, light brown to grey, with red-brown margin slightly to not raised and occasionally calloused; parasitic wasp larva with host larva or an exit hole was frequently observed in cavities within the lesion which caused the centre of the lesion to rise. Pseudothecia hypophyllous, often aggregated in a distinct band surrounding the raised centre of the lesion, black, globose, occasionally periphyses present, 50–110 μm diameter. Asci fasciculate, bitunicate, aparaphysate, subsessile, 8–spored, obclavate to cylindrical, straight or curved, 30–55 $\times$ 7.5–14 μm. Ascospores, 2–3–seriate, hyaline, 1-septate, ends obtusely rounded, slightly constricted at the median septum or not so, widest in the middle of the apical cell, slightly tapered towards the basal end (10)−14–17–(20) $\times$ (3)−4–(5) μm. Pycnidia hypophyllous, often aggregated in a distinct band surrounding the raised centre of the lesion, occasionally dispersed between pseudothecia, globose, 32–75 μm diam. wall 2–3 layers of dark brown textura angularis. Conidiogenous cells verruculose, pale brown, ampulliform to dolliform, enteroblastic and proliferating percurrently. Conidia ellipsoidal, lightly pigmented pale brown, apex subobtuse, base truncate, generally widest at or below the median, finely verruculose (7.5)−9–(12) $\times$ 2.5–3–(5) μm; basal marginal frill present.

Ascospore germination: Ascospores of *M. vespa* germinate at 22°C after 24 h on MEA from the apices of the both cells or one cell first (Fig. 4), parallel to the long axis of the spore. Spores do not darken at germination.

Ascospore cultures: Single spore cultures are relatively fast growing, up to 24 mm diameter in 1 month. Submerged mycelium thin and grey-green to brown, often becoming dendritic towards the outer margin. Aerial hyphae are light grey or grey-olivaceous to



Figs 2, 3, 4. Mycosphaerella vespa. Fig. 2. Asci containing ascospores; Fig. 3. Ascospores; Fig. 4. Ascospore germination at 22°C after 24 h on malt extract agar (Bar, 10 μm); Fig. 5. *Coniothyrium ovatum* conidia in vitro (Bar, 10 μm)

white and fluffy, becoming sparser towards the edge of cultures. Pycnidial structures scattered throughout and producing conidia after 2–4 weeks storage in the dark on MEA at 22°C (5)−8–(12) × 2.5–3–(5) μm.
Host: *E. globulus*
Distribution: South-eastern Victoria and Tasmania, Australia
Specimens examined: *M. vespa* on *E. globulus*, Swansea, Tasmania, A. W. Milgate, 27 May 1998 (VPRI 22377); *M. vespa* and *C. ovatum* on *E. globulus*, Bruny Island, Tasmania, C. Mohammed, 20 Jan. 1998 (VPRI 22378a and b); *M. vespa* and *C. ovatum* on *E. globulus*, Scamander, Tasmania, A. W. Milgate, 16 Sep. 1998 (VPRI 22379a and b); *M. vespa* on *E. globulus*, Hobart, Tasmania, A. W. Milgate, 9 Apr. 1998 (VPRI 22388); *C. ovatum* on *Eucalyptus dives*, near Bendigo, Victoria, 17 May 1983, H. J. Swart (DAR 49461).
Note: Twice *M. vespa* was isolated from lesions showing no obvious signs of insect involvement. On adult foliage *M. vespa* was only found in the presence of the parasitic wasp. *Coniothyrium ovatum* was found in lesions containing *M. vespa* at three locations and twice without *M. vespa*. Infection studies have shown the ascospores of *M. vespa* to be infective and the conidia of *C. ovatum* to be noninfective (Milgate, Vaillancourt and Mohammed, unpublished data).

4 Discussion

The most frequently encountered *Mycosphaerella* species in Tasmanian juvenile plantations of *E. nitens* and *E. globulus* are *M. nubilosa* and *M. cryptica*, although these species vary in host specificity. *Mycosphaerella cryptica* and *M. nubilosa* were very common throughout the state on *E. globulus* and *M. cryptica* was frequently found on *E. nitens*. *Mycosphaerella nubilosa* was not observed on *E. nitens* during the survey. A similar pattern for *M. cryptica* and *M. nubilosa* has been observed in Victoria, southern Australia (Keane, personal comm. 1999). *Mycosphaerella tasmaniensis* displayed some regional specificity. This species was found on both *E. globulus* and *E. nitens* but only in the northern regions of Tasmania. The most frequently isolated species, *M. cryptica* and *M. nubilosa*, also had a higher incidence of severe infections. These two species and *M. tasmaniensis* appear to have the greatest potential to damage juvenile eucalypt plantations in Tasmania.

There were small differences in the morphology of *M. nubilosa*, *M. cryptica* and *M. grandis* to reported dimensions for these species but these were not significant. Sequencing of the 5.8S gene and internal transcribed spacer regions of their nuclear rDNA has shown that Tasmanian isolates identified as belonging to *M. nubilosa*, *M. cryptica* or *M. grandis* are identical to isolates of the same species from Victoria and New Zealand (Milgate, Vaillancourt and Mohammed, unpublished data). Isolates of *M. tasmaniensis* were similar to its type description (CROUS et al. 1998) but this survey constitutes the first record of *M. tasmaniensis* on *E. globulus*.

In the last decade the reviewing of taxonomic records and the examination of new material have led to the description of 23 new *Mycosphaerella* species on eucalypt foliage, seven of these described from Australia. Previous taxonomic records for *Mycosphaerella* species in Tasmania however, proved reliable. Apart from the recent description of *M. tasmaniensis* (CROUS et al. 1998) just prior to the survey, no new species were described as a result of the survey and only one new record for Tasmania (*M. vespa*) was established. Six (including anamorphs) out of the seven species already reported from Tasmania were detected during the survey. The species recorded for Tasmania but not found during this survey was *Mycosphaerella delegatensis* Park and Keane. *Mycosphaerella delegatensis* has only been recorded on *Eucalyptus delegatensis* Baker and *Eucalyptus obliqua* L'Her. in Tasmania. The latter tree species were not examined during the survey.

Table 3. **A comparison of dimensions reported by various workers and those obtained during this study**

Species	Asci (μm)	Pseudothecia (diameter μm)	Ascospore (length × width μm)	Ascospore germ pattern[1]	Source
M. cryptica	35–62 × 10–16	80–115	10–17 × 3.5–5-(7.5)	A	This study
	35–59 × 7–15	up to 130	9–17.5 × 2–5.5	A	Crous (1998)
	40 × 12–15	up to 130	10–12 × 2–3	ND	Hansford (1956)
M. nubilosa	32–55 × 7–15	55–110	(12.5)−14–16(18) × (5)−6(7.5)	C	This study
	30–68 × 9–18	40–150	(11–)13–16 × 3–3.5(− 4.5)	C	Crous (1998)
	50 × 18	100–156	12–14 × 2.5–3	ND	Hansford (1956)
M. tasmaniensis	30–52 × 10–17	35–110	(9)10–13-(15) × (3)−4–(5)	I	This study
	30–40 × 7–11	80–110	(10)−11–12(13) × (2.5)3(4)	I	Crous (1998)
M. grandis	25–50 × 7.5–11	45–82	(10)−11–(15) × (4)−5–(7.5)	N and L	This study
	35–37.5 × 10	60–70	10.5–14.5(12.3) × 3–4.5	N	Carnegie and Keane (1994)
M. vespa	30–55 × 7.5–14	50–110	(10)14–17(20) × (3)−4–(5)	C	This study
	40–52 × 7–10.5	73–130	9.5–16.5 × 2.5–4	C and I	Carnegie and Keane (1998)
C. ovatum§	–	32–75	(7.5)-9–(12) × (2.5)−3–(5)	–	This study
	–	up to 80	(6-)7–9(− 11) × 3–3.5(− 4)	–	Crous (1998)
	–	40–70(80)	(6)7–11 × 3–4.5(5)	–	Swart (1986)
S. eucalypticola§	–	65–120	18–30 × 7–12	–	This study
	–	up to 120	19–31 × 6–12	–	Crous (1998)
	–	100–150	20–26 × 9–11	–	Swart and Walker (1988)
S. eucalyptorum§	–	100–125	25–35 × 4–7	–	This study
		up to 120	25–49 × 5–10	–	Crous (1998)

[1]Ascospore germination patterns taken from Crous (1998).

Type A: Ascospores germinate with germ tubes perpendicular to the long axis of the spore. Germination occurs from only one cell, and germ tube can originate at or near the apex. Spores are naturally constricted, and become slightly more so upon germination, are olivaceous, but stay smooth.

Type C: Ascospores germinate from both ends, with germ tubes parallel to the long axis of the spore. Slight constriction at the median septum.

Type I: Germ tubes grow parallel to the long axis of the spore, slight constriction develops at the median septum, and lateral branches develop along the axis, 24–28 h after germination.

Type L: Ascospores germinate from both ends, and germ tubes grow perpendicular to the long axis of the spore. Ascospores become distorted, constricted at the septum, medium brown, smooth to finely verruculose. Sometimes germ tubes spiral around the longitudinal axis.

Type N: Mostly darkly pigmented verruculose analogue of type A. After 48 h, germinated ascospores resemble type H.

Type ND: Not determined. § – indicates measurements are of conidia and pycnidia. Extremes given in parentheses.

As with the type description, *M. vespa* collected in Tasmania on the juvenile and adult foliage of *E. globulus* had similarities with several other *Mycosphaerella* species; *Mycosphaerella gregaria* Carnegie and Keane, *Mycosphaerella molleriana* (Thüm.) Lindau and *M. nubilosa* (three species which germinate from both ends of the ascospore) and *M. cryptica* with circular to irregular lesions (CARNEGIE and KEANE 1998). However association of lesions with wasps and a clear link to the anamorph *C. ovatum* distinguished Tasmanian isolates designated *M. vespa* as a separate species, a distinction confirmed by molecular analyses (Milgate, Vaillancourt and Mohammed, unpublished data).

Leaf lesions of *M. vespa* in Tasmania were slightly larger (up to 7 mm diameter) than those of the type description (up to 5 mm diameter) and on two occasions *M. vespa* was isolated from lesions with no evidence of insect exit holes. Pseudothecia were smaller in the Tasmanian collection, 50–11 μm diameter, compared with 73–110 μm diameter in the type description, often aggregated in a band surrounding the raised centre of lesions and were predominantly hypophyllous, occasionally interspersed with pycnidia of *C. ovatum*. Single ascospore cultures of *M. vespa* produced pycnidia and conidia of *C. ovatum*. In contrast CARNEGIE and KEANE (1998) reported that the pseudothecia of *M. vespa* were scattered and amphigenous. These authors did not observe pycnidia of *C. ovatum* either on field specimens or single ascospore cultures. An additional feature occasionally noted on the Tasmanian collection was the presence of periphyses. A third germ tube described in the type specimens was not seen in the Tasmanian collection. Germinating spores were also observed to often have one germtube more advanced in growth than the other (Fig. 4).

Anamorph genera linked to *Mycosphaerella* species are important in the delimitation of species and perhaps eventually the division of the genus into subgenera (CROUS 1998). Over 40 anamorph genera have been linked to *Mycosphaerella* from both the coelomycetes and hyphomycetes (CROUS 1998). Prior to this article eight of these have been linked to 18 *Mycosphaerella* species recorded on eucalypts. This is the first time a teleomorph/anamorph link between *Mycosphaerella* and *Coniothyrium* Corda has been unequivocally demonstrated. CORLETT (1991) reported several *Coniothyrium* species as possible anamorphs of *Mycosphaerella* although CROUS (1998) later refuted this association given the known link of *Coniothyrium* with *Leptosphaeria* Ces. and De Not teleomorph species. Now that a *Mycosphaerella* and *Coniothyrium* link has been firmly established, the possibility that two other *Coniothyrium* species on eucalypt foliage, *Coniothyrium eucalypticola* B. Sutton and *Coniothyrium kallangurense* B. Sutton and Alcorn have *Mycosphaerella* teleomorphs should not be discounted.

The *C. ovatum* collected in Tasmania appears conspecific with that of the *C. ovatum* described by SWART (1986). Crous (personal comm.) considers that *Coniothyrium* species on eucalypts may belong to *Phaeophleospora* Rangel because of the known link between *Coniothyrium* and *Leptosphaeria* (CROUS 1998) and also because of the characteristic of conidiogenous cells. *Coniothyrium* has hyaline holoblastic conidiogenous cells (SUTTON 1980). We examined the type material of *C. ovatum* (DAR 49461) and found conidiogenous cells were brown, verruculose and proliferating percurrently as found in *Phaeophleospora*. The presence of pigmented conidiogenous cells in the type material of *C. ovatum* indeed may indicate that the inclusion of *C. ovatum* in *Phaeophleospora* is appropriate. However sequence data of the ITS region of *Mycosphaerella* species and their anamorphs on eucalypts indicate that *C. ovatum* and *Phaeophleospora eppicoccoides* (Cook and Massee) Crous, F.A. Ferreira and B. Sutton may not be closely related (Milgate, Vaillancourt and Mohammed, unpublished data) suggesting that further work is required to clarify this situation.

Some differences were noted between the Tasmanian specimens of *C. ovatum* and the type specimen of SWART (1986). Leaf lesion size was larger in the Tasmanian collections, up to 7 mm diameter compared to 1 mm diameter given in the type description. Pycnidia development was predominantly hypophyllous and not amphigenous as described in the

type specimen. Pycnidia diameters were slightly smaller in the Tasmanian collection, up to 75 μm diameter compared with 80 μm diameter. Conidia produced in culture did not always appear to be finely verruculose. Conidial dimensions were slightly larger *in vivo* (7.5)−9–(12) × 2.5–3–(5) μm compared with *in vitro* (5)−8–(12) × 2.5–3–(5) μm but not significantly different from the type specimen 7–11 × 3–4.5 (5) μm.

The *C. ovatum* specimens collected during this survey appear most similar to *Coniothyrium* specimens described by CROUS et al. (1988) from South Africa. Compared with the type material both the Tasmanian and South African specimens have larger lesions, predominantly hypophyllous pycnidia and slightly larger conidia. However, *C. ovatum* recorded in New Zealand by RIDLEY (1995) has spores which are larger again 10.6–13.6(−14.3) × 5.3–6.1 μm. Conidia produced by isolates from South African specimens were noninfective in artificial inoculations (CROUS et al. 1989). Inoculation tests also demonstrated that the conidia of *C. ovatum* collected in Tasmania were noninfective (Milgate, Vaillancourt and Mohammed, unpublished data). CROUS (1998) considered it likely that the specimens he examined in South Africa were not *C. ovatum* although very similar in morphology.

In conclusion we have shown that there are at least seven *Mycosphaerella* species (including anamorphs) found on the *E. globulus* and *E. nitens* plantations of Tasmania. *Mycosphaerella tasmaniensis* is recorded here for the first time on *E. globulus* and *M. vespa* as a new record in Tasmania. The coelomycete *C. ovatum* is confirmed here for the first time linked to *M. vespa*. This is also the first time a link between these two genera has been confirmed. Although differences were observed between Tasmania and Victorian collections of *M. vespa* (the most significant being its association with the anamorph *C. ovatum*) these differences are not sufficient however, to justify the description of Tasmanian isolates as a new species until further morphological and molecular comparisons can be completed. These further taxonomic investigations should include a comparison of *C. ovatum* from South Africa, New Zealand and Tasmania.

This detailed taxonomic survey of *Mycosphaerella* species present in young eucalypt plantations in Tasmania constitutes the first stage in the process of developing accurate methods for assessing economic injury levels and developing control strategies relevant to industry requirements.

Acknowledgements

This work forms part of a PhD project which is supported by an ARC, APRI grant. The industry partner of this program is North Forest Products. I would like to take this opportunity to thank both for their financial support and North in providing field assistance. We are grateful to Dr Pedro CROUS, Dr Margaret DICK and Dr Geoff RIDLEY for providing critical comments on an early draft.

Résumé

Les espèces de Mycosphaerella dans les plantations d'Eucalyptus globulus et Eucalyptus nitens en Tasmanie (Australie)

Le genre *Mycosphaerella* Johanson comprend de nombreuses espèces parasites capables d'avoir un fort impact sur la croissance des espèces sensibles d'eucalyptus. Le manque de connaissance surs les espèces présentes en Tasmanie et les risques potentiels qu'elles représentent pour les plantations industrielles, a justifié la présente étude sur *Eucalyptus globulus* et *E. nitens*. Trente-six plantations et 5 sites de bord de route à *E. globulus* et *E. nitens* ont été étudiés. Cinq espèces de *Mycosphaerella* et trois espèces anamorphes ont été isolées et indentifiées en Tasmanie: *Mycosphaerella nubilosa*, *Mycosphaerella cryptica*, *Mycosphaerella tasmaniensis*, *Mycosphaerella grandis*, *Mycosphaerella vespa*, *Coniothyrium ovatum*, *Sonderhenia eucalypticola*, et *Sonderhenia eucalyptorum*. Les espèces les plus fréquemment isolées, associées à une infection et à un impact plus élevé étaient *M. cryptica* et *M. nubilosa*. Ces deux espèces apparaissent comme les plus dangereuses, potentiellement, pour les jeunes plantations d'eucalyptus en Tasmanie. La liaison entre *M. vespa* et *C. ovatum* est décrite pour la première fois.

Zusammenfassung

Vorkommen von Mycosphaerella-Arten an Eucalyptus globulus und Eucalyptus nitens in Tasmanien, Australien

Die Gattung *Mycosphaerella* enthält zahlreiche bedeutende Eucalyptus-Pathogene, deren Vorkommen in Tasmanien bisher nicht untersucht wurde. Um das von *Mycosphaerella*-Infektionen ausgehende Risiko für die Holzproduktion in diesem Gebiet abzuschätzen, wurde das Vorkommen dieser Pilze in Plantagen von *Eucalyptus globulus* und *Eucalyptus nitens* erfasst. Insgesamt wurden 36 Standorte in Plantagen und entlang von Strassenrändern untersucht. Es wurden fünf *Mycosphaerella*-Arten und drei Arten von damit assoziierten Nebenfruchtformen nachgewiesen: *Mycosphaerella nubilosa*, *Mycosphaerella cryptica*, *Mycosphaerella tasmaniensis*, *Mycosphaerella grandis*, *Mycosphaerella vespa, Coniothyrium ovatum*, *Sonderhenia eucalypticola* und *Sonderhenia eucalyptorum*. Die beiden häufigsten und zugleich pathogensten Arten waren *M. cryptica* und *M. nubilosa*, die das grösste Schadpotential in jungen Eucalyptusplantagen in Tasmanien aufweisen. *Coniothyrium ovatum* wurde erstmals als Anamorph von *M. vespa* nachgewiesen.

References

CARNEGIE, A. J.; KEANE, P. J., 1994: Further *Mycosphaerella* species associated with leaf diseases of *Eucalyptus*. Mycol. Res. **98,** 413–418.

CARNEGIE, A. J.; KEANE, P. J., 1998: *Mycosphaerella vespa* sp. nov. from diseased *Eucalyptus* leaves in Australia. Mycol. Res. **102,** 1274–1276.

CORLETT, M., 1991: An annotated list of the published names in *Mycosphaerella* and *Sphaerella*. Mycol. Mem. **18,** 1–328.

CROUS, P. W., 1998: *Mycosphaerella* spp. and Their Anamorphs Associated with Leaf Spot Diseases of *Eucalyptus*. The Mycological Society of America. Mycological Memoir No. 21. St. Paul, Minnesota: APS Press.

CROUS, P. W.; KNOX-DAVIES, P. S.; WINGFIELD, M. J., 1988: *Phaeoseptoria eucalypti* and *Coniothyrium ovatum* on *Eucalyptus* spp. in South Africa. Phytophylactica **20,** 337–340.

CROUS, P. W.; KNOX-DAVIES, P. S.; WINGFIELD, M. J., 1989: Infection studies with *Phaeoseptoria eucalypti* and *Coniothyrium ovatum* on *Eucalyptus* spp. S. Afr. For. J. **149,** 30–35.

CROUS, P. W.; WINGFIELD, M. J., 1996: Species of *Mycosphaerella* and their anamorphs associated with leaf blotch disease of *Eucalyptus* in South Africa. Mycologia **88,** 441–458.

CROUS, P. W.; WINGFIELD, M. J., 1997: *Colletogloeopsis*, a new coelomycete genus to accommodate anamorphs of two species of *Mycosphaerella* on *Eucalyptus*. Can. J. Bot. **75,** 667–674.

CROUS, P. W.; WINGFIELD, M. J.; PARK, R. F., 1991: *Mycosphaerella nubilosa*, a synonym of *M. molleriana*. Mycol. Res. **95,** 628–632.

CROUS, P. W.; WINGFIELD, M. J.; MOHAMMED, C.; YUAN, Z. Q., 1998: New foliar pathogens of *Eucalyptus* from Australia and Indonesia. Mycol. Res. **102,** 527–532.

GANAPATHI, A.; CORBIN, J. B., 1979: *Colletogloeum nubilosum* sp. nov., the imperfect state of *Mycosphaerella nubilosa* on *Eucalyptus* in New Zealand. Trans. Br. Mycol. Soc. **72,** 237–244.

HANSFORD, C. G., 1956: Australian fungi. III New species and revisions (cont'd). Proc. Lin. Soc. N.S.W. **81,** 23–51.

PARK, R. F.; KEANE, P. J., 1984: Further *Mycosphaerella* species causing leaf diseases of *Eucalyptus*. Trans. Br. Mycol. Soc. **83,** 93–105.

RIDLEY, G. S., 1995: Mycological Records 3: *Coniothyrium ovatum* Swart. N. Z. J. For. Sci. **25 (1),** 105–106.

SUTTON, B. C., 1980 : The Coelomycetes. Kew, UK: Commonwealth Mycological Institute.

SWART, H. J., 1986: Australian leaf-inhabiting fungi XXI. *Coniothyrium* on *Eucalyptus*. Trans. Br. Mycol. Soc. **86,** 494–496.

SWART, H. J.; WALKER, J., 1988: Australian leaf-inhabiting fungi. XXVIII. *Hendersonia* on *Eucalyptus*. Trans. Br. Mycol. Soc. **90,** 633–641.